ENGINEERED TGF-BETA MONOMERS AND THEIR USE FOR INHIBITING TGF-BETA SIGNALING

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is the continuation of U.S. patent application Ser. No. 16/461,747, filed May 16, 2019, which is the U.S. National Stage of International Application No. PCT/US2017/062233, filed Nov. 17, 2017, published in English under PCT Article 21(2), which claims the benefit of U.S. Provisional Application No. 62/423,920, filed Nov. 18, 2016. The above-listed applications are herein incorporated by reference in their entirety.

FIELD

[0002] This disclosure concerns recombinant transforming growth factor (TGF)-β monomers modified to inhibit dimerization while retaining the capacity to bind the high affinity TGF-β type II receptor (TβRII). This disclosure further concerns use of the recombinant TGF-β monomers to inhibit TGF-β signaling.

ACKNOWLEDGMENT OF GOVERNMENT SUPPORT

[0003] This invention was made with government support under grant number GM058670, awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

[0004] TGF-β is a multifunctional cytokine with diverse biological effects on cellular processes, including cell proliferation, migration, differentiation, and apoptosis. The three mammalian TGF-β isoforms, TGF-β1, -β2 and -β3, exert their functions through a cell surface receptor complex composed of type I (TβRI) and type II (TβRII) serine/threonine kinase receptors. Receptor activation induces both SMAD proteins and other downstream targets, including Ras, RhoA, TAK1, MEKK1, PI3K, and PP2A, to produce the full spectrum of TGF-β responses (Roberts and Wakefield, *Proc Natl Acad Sci USA* 100:8621-8623, 2003; Derynck and Zhang, *Nature* 425:577-584, 2003; Massagué, *Cell* 134:215-230, 2008).

[0005] TGF-β proteins are known to promote the progression of fibrotic disorders and certain types of cancer. In the context of fibrotic disorders, TGF-β potently stimulates the expression of extracellular matrix (ECM) proteins. Dysregulation of the ECM remodeling can lead to pathological fibrosis. The role of TGF-β in cancer is multi-faceted. TGF-β isoforms, TGF-β1, -β2 and -β3 are also known to suppress host immune surveillance and to stimulate epithelial-to-mesenchymal transitions, which drive cancer progression and metastasis.

SUMMARY

[0006] Described herein are engineered TGF-β monomers that are capable of blocking TGF-β signaling. The engineered monomers inhibit TGF-β signaling by preventing TGF-β dimerization and recruitment of TβRI.

[0007] Provided herein is a recombinant TGF-β monomer that includes a cysteine to serine substitution at amino acid residue 77; a deletion of amino acid residues 52-71; and at least one amino acid substitution that increases the net charge of the monomer. In some embodiments, the TGF-β monomer further includes at least one amino acid substitution that increases affinity of the TGF-β monomer for TGF-β type II receptor (TβRII). The TGF-β monomer can be, for example, a TGF-β2, TGF-β1 or TGF-β3 monomer, such as a human, rat, mouse or other mammalian TGF-β2, TGF-β1 or TGF-β3 monomer.

[0008] Fusion proteins that include a TGF-β monomer and a heterologous protein are also provided. Further provided are compositions that include a recombinant TGF-β monomer or fusion protein disclosed herein and a pharmaceutically acceptable carrier, diluent, or excipient.

[0009] Further provided are methods of inhibiting TGF-β signaling in a cell by contacting the cell with a recombinant TGF-β monomer, fusion protein or composition disclosed herein. In some embodiments, the method is an in vitro method. In other embodiments, the method is an in vivo method that includes administering the recombinant TGF-β monomer, fusion protein or composition to a subject having a disease or disorder associated with aberrant TGF-β signaling.

[0010] Also provided are nucleic acid molecules and vectors that encode a recombinant TGF-β monomer disclosed herein. Further provided are isolated cells, such as isolated T lymphocytes, that comprise the recombinant TGF-β monomer-encoding nucleic acid molecule or vector.

[0011] Methods of treating a disease or disorder associated with aberrant TGF-β signaling in a subject by administering to the subject an isolated cell (such as a T cell) comprising the disclosed nucleic acids or vectors are further provided.

[0012] The foregoing and other objects, features, and advantages of the invention will become more apparent from the following detailed description, which proceeds with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] FIGS. **1**A-**1**F: Structure of the TGF-β signaling complex and sequences of the engineered TGF-β variants lacking the interfacial α-helix. (FIG. **1**A) Cartoon representation of the TGF-β signaling complex formed between the human TGF-β3 homodimer and the extracellular ligand binding domains of the human TGF-β type I and type II receptors, TβRI and TβRII (PDB 2PJY) (Groppe et al., *Mol Cell* 29, 157-168, 2008). The disulfide bonds, including the single inter-chain disulfide connecting the TGF-β monomers, are shown. The TGF-β monomers are described as curled left hands, with the heel formed by a 3-1/2 turn α-helix (α3) and the four fingers formed by the β-strands that extend from the cystine knot that stabilizes each monomer. (FIG. **1**B) Expanded view illustrating packing interactions formed by hydrophobic residues that emanate from the heel α-helix of one TGF-β3 monomer with hydrophobic residues from the palm region of the opposing TGF-β3 monomer. (FIG. **1**C) Expanded view illustrating ionic, hydrogen bonding, and hydrophobic interactions that stabilize TβRI at the composite interface formed by both monomers of TGF-β3 and TβRII. (FIG. **1**D) Sequence alignment of TGF-β1, β2, and -β3 with monomeric variants in which Cys77, which normally forms the inter-chain disulfide bond, is substituted with serine (mTGF-β2 and mTGF-β3) or mini monomeric variants in which Cys77 is substituted with serine, residues 52-71 have been deleted, and 2 or 3 addi-